

CLAIMS



We claim:

1. A method for introducing a nucleic acid into a *Rhodococcus* bacterium, comprising:
culturing a first strain of *Rhodococcus* bacterium transformed with a plasmid comprising a nucleic acid sequence that enables conjugative transfer of the plasmid with a second strain of *Rhodococcus* bacterium for a length of time under conditions such that conjugative transfer occurs between said first and second strains of bacterium, whereby said plasmid is transferred from said first strain of bacterium to said second strain of bacterium.
2. The method of claim 1, wherein said plasmid further comprises a gene construct.
3. The method of claim 2, wherein said gene construct encodes a protein to be expressed in said second strain of bacterium.
4. The method of claim 3, wherein said gene construct further comprises at least one promoter suitable for the expression of a gene in a *Rhodococcus* bacterium.
5. The method of claim 4, wherein said gene is expressed in said second strain of *Rhodococcus* bacterium following said transferring.
6. The method of claim 3, wherein said plasmid comprises a mutant gene.
7. The method of claim 3, wherein said plasmid comprises a fragment of a gene.
8. The method of claim 1, wherein said plasmid or a portion thereof is integrated into the genome of said second strain of bacterium.
9. The method of claim 8, wherein said integration occurs by homologous recombination.
10. The method of claim 8, wherein the replication of said plasmid is temperature sensitive.

11. The method of claim 10, wherein said plasmid further comprises (a) SEQ ID NO: 2; (b) a sequence that hybridizes with (a) under stringent hybridization conditions; and (c) a sequence that is complementary to (a) or (b).
12. The method of claim 10, further comprising the step of selecting a bacterium of said second strain that has integrated said plasmid or a portion thereof into its genome by elevating the temperature of the culture.
13. A method for disrupting or knocking out a gene in a *Rhodococcus* bacterium, comprising: culturing a first strain of *Rhodococcus* bacterium transformed with a plasmid comprising a nucleic acid sequence that enables conjugative transfer of the plasmid and a nucleic acid sequence that serves to disrupt or knock out said gene with a second strain of *Rhodococcus* bacterium for a length of time under conditions such that conjugative transfer occurs between said first and second strains of bacterium, whereby said plasmid is transferred from said first strain of bacterium to said second strain of bacterium and said sequence that serves to disrupt or knock out said gene is integrated into the genome of said second second strain of bacterium.
14. The method of claim 13, wherein said integration occurs by homologous recombination.
15. The method of claim 13, wherein said nucleic acid sequence that enables conjugative transfer of the plasmid is selected from the group consisting of: (a) a nucleic acid sequence comprising the region involved in conjugal transfer containing origin of Transfer (oriT) sequence; (b) a nucleic acid sequence comprising the origin of Transfer (oriT) sequence; (c) a nucleic acid sequence that hybridizes with (a) or (b) under stringent hybridization conditions; and (d) a nucleic acid sequence that is complementary to (a), (b) or (c).
16. A method for selecting from a culture a recipient *Rhodococcus* bacterium that has integrated a plasmid or a portion thereof into its genome comprising the use of temperature selection.
17. The method of claim 16, comprising conjugatively transferring from a donor *Rhodococcus* bacterium to a recipient *Rhodococcus* bacterium that can grow above a

minimum temperature a plasmid that cannot replicate above said minimum temperature; elevating the temperature of the culture above said minimum temperature for a sufficient time to prevent replication of said plasmid and survival of said donor *Rhodococcus* bacterium, whereby only a recipient *Rhodococcus* bacterium that has incorporated said plasmid into its genome survives such temperature elevation.

18. The method of claim 17, wherein said plasmid comprises (a) the minimal replicon sequence; (b) a sequence that hybridizes with (a) under stringent hybridization conditions; and (c) a sequence that is complementary to (a) or (b).
19. The method of claim 17, wherein said minimum temperature is 30°C and the temperature is elevated to 37°C.
20. The method of claim 18, wherein said donor *Rhodococcus* bacterium is of the strain *Rhodococcus* B264-1 and said recipient *Rhodococcus* bacterium is of the strain *Rhodococcus* I24.